Nodule-specific and Nodule-induced Monosaccharide Transporters (MSTs) in *Medicago truncatula*.

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Abstract

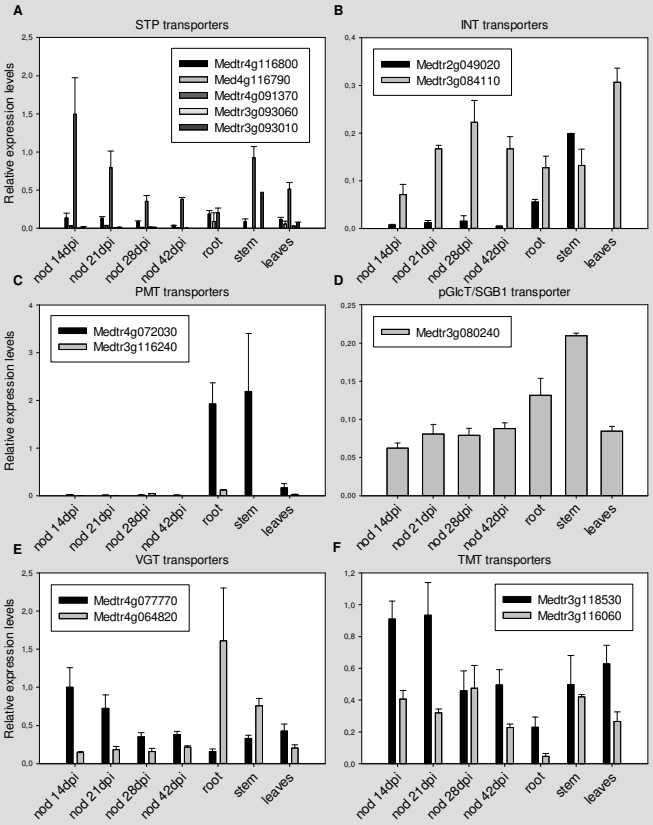
Legumes are cornerstones of sustainable agriculture, as the symbiotic relation they form with soil bacteria, called rhizobia, results in nitrogen fixation (symbiotic nitrogen fixation – SNF). SNF takes place inside new plant organs that develop for this reason, the legume root nodules. Plant cell membrane transporters are essential for nutrient exchange between legumes and rhizobia, facilitating the appropriate conditions for nodule metabolism and for being potential sites of SNF regulation. We have used the model symbiotic system of *Medicago truncatula* - *Sinorhizobium meliloti* to identify plant genes involved in carbon transport in the nodule. *M. truncatula* is an excellent candidate for such studies, due to the available databases regarding the sequencing of the genome (http://mtgea.noble.org/v2, http://www.medicago.org/genome, NCBI), the expression of genes (http://mtgea.noble.org/v2) and the active metabolic pathways (http://www.genome.jp/kegg/pathway.html). *In silico* analysis was conducted to identify *M. truncatula* Monosaccharide Transporters (MSTs) that are nodule-induced or nodule-specifically expressed. Here, we present data concerning the phylogenetic taxonomy of these transporters, their gene structure, and a prediction of the topology/secondary structure for the corresponding encoded protein. Furthermore, total RNA was extracted from different organs and nodule developmental stages of *M. truncatula,* and the expression levels of these genes, analysed by RT-qPCR, is depicted. This work represents the starting point for the elucidation of the identified MSTs' exact physiological and biochemical role during SNF using the available reverse genetic resources for *M. truncatula*.

The authors thank the Ministry of Education, Lifelong Learning and Religious Affairs for the financial assistance provided. This work was performed within the grant program ARISTEIA II, co-funded by the European Union – European Social Fund & National Resources.
One of the authors, F. Komaitis thanks the Onassis Foundation for the scholarship granted to him.

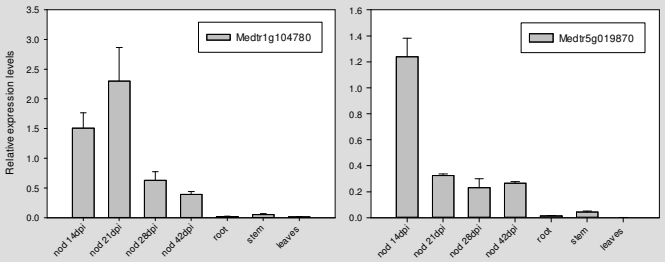
Materials and Methods

- *In silico* analysis in Noble Foundation's *M. truncatula* Expression Atlas (MtGEA) and JCVI: Medicago public databases.
- BioEdit and Mega 5.2 programs were used for the construction of the MSTs dendrogram.
- TMHMM Server v. 2.0 for the prediction of transmembrane helices in proteins.
- RNA isolation and cDNA synthesis.
- Design of gene-specific primers.
- qPCR to estimate gene expression levels.

Results



Graph 1: Relative expression levels of the examined STP (A), INT (B), PMT (C), pGlcT/SGB1 (D), VGT (E), and TMT (F) transporters in different nodule developmental stages and non-symbiotic organs. Bars represent means +/- SE of independent biological repeats (n=3).



Graph 2: Relative expression levels of Medtr1g104780 and Medtr5g019870 in different nodule developmental stages and in different non-symbiotic organs. Bars represent means +/- SE of independent biological repeats (n=3).

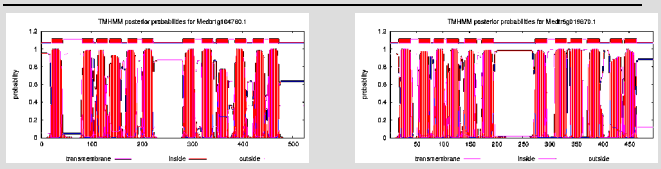
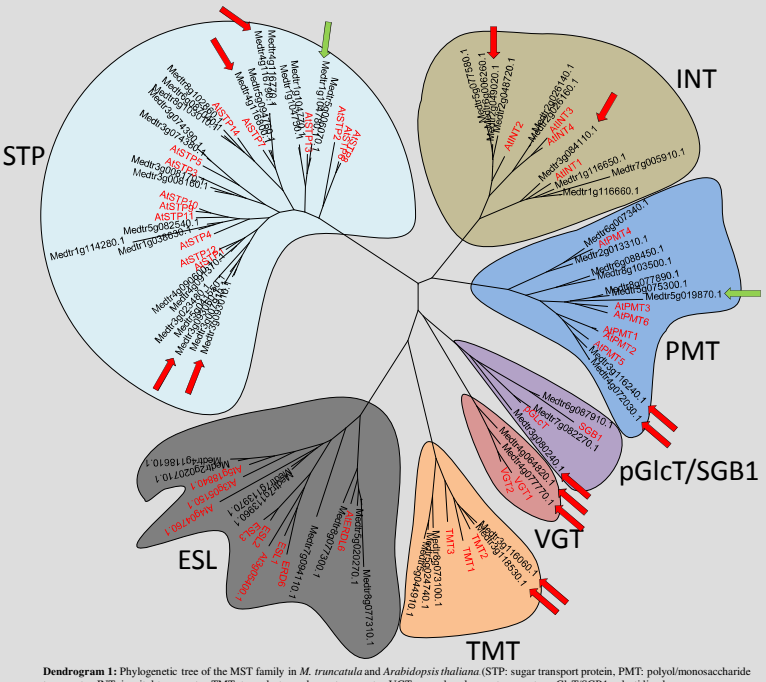


Figure 1: Predicted secondary structure of Medtr1g104780 & Medtr5g019870.



Dendrogram 1: Phylogenetic tree of the MST family in *M. truncatula* and *Arabidopsis thaliana* (STP: sugar transport protein, PMT: polyol/monosaccharide transporter, INT: inositol transporter, TMT: tonoplast membrane transporter, VGT: vacuolar glucose transporter, pGlcT/SGB1: plastidic glucose transporter/suppressor of G Protein Beta 1, ESL: early-responsive to dehydration six-like).

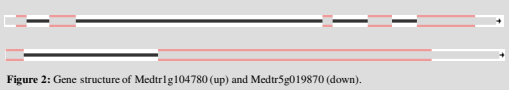


Figure 2: Gene structure of Medtr1g104780 (up) and Medtr5g019870 (down).

Conclusions

- Phylogenetic taxonomy of the 62 MSTs identified in the *M. truncatula* genome and grouping in all known subfamilies (STP:25, INT:10, PMT:9, pGlcT/SGB1: 3, VGT: 2, TMT: 5, ESL: 8)
- Most of the MSTs showed different levels of expression in different nodule developmental stages and different non-symbiotic organs.
- Medtr4g072030 and Medtr3g116240 showed high expression levels in root and stem.
- qRT-PCR results verified the nodule specificity in the expression of the two sugar transporters [Medtr1g104780 (STP) and Medtr5g019870 (PMT)], as found in MtGEA.
- Predicted secondary structures of the two nodule-specific transporters are presented along with gene structure.
- The large number of the MSTs and the expression of these genes in all plant tissues indicates their significant role in plant growth and their physiological and biochemical significance during SNF.
- Ongoing work:

  a) topology studies regarding the localization of the mRNAs of Medtr1g104780 and Medtr5g019870 and their corresponding proteins through expression studies of transporter-YFP hybrids in nodules.

  b) biochemical characterization of the transport activity after their expression in the *Saccharomyces cerevisiae* system.

  c) phenotypic characterization of *M. truncatula Tnt1* mutant lines for the target genes.

European Union
European Social Fund
OPERATIONAL PROGRAMME EDUCATION AND LIFELONG LEARNING
MINISTRY OF EDUCATION & RELIGIOUS AFFAIRS
MANAGING AUTHORITY
NSRF 2007-2013
EUROPEAN SOCIAL FUND
Co-financed by Greece and the European Union